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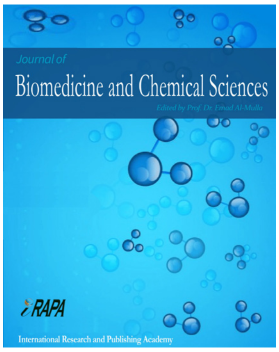

Application of Cloud Point in Spectrophotometric Determination of Drugs: Overview

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ABSTRACT

Cloud Point Extraction (CPE) technique depends on the preconcentration of material in aqueous medium, and it is known that the surfactants in the mentioned technique could concentrate these materials. The CPE technique receives great attention because of the simplicity and speed of the method, and the extraction process is beneficial by improving the experimental conditions by adjusting the acidity function, temperature, type of electrolyte, extraction time and surfactant concentration. The surfactant leads to the phase separation of the so-called surfactant-rich phase and the surfactant aqueous phase. The comparison of the maximum temperature with the critical temperature is called the cloud point temperature which will be Then the substance to be analyzed is concentrated and the preconcentration factor is high. This article describe the applications of CPE method in the determination of different drugs using deferent reactions and various reagents.

INTRODUCTION

In 1978, Watanabe and Tanaka were the first chemists to introduce CPE as a technique of green extraction, which uses a surfactant as an extractant rather than organic solvent (Watanabe & Tanaka, 1978; Ghasemi & Kaykhaii, 2016). An analytical method with tremendous potential to increase detection limits is separation and pre-concentration. There are many advantages to using CPE as a pre-concentration process. Some of features include speed, safety, and low cost. CPE is a simple method that concentrates a wide range of analytes with excellent recoveries and high concentration factors. Results with CPE are equivalent to those acquired using other methods of separation. Species that interact with the micellar system are therefore either directly (usually hydrophobic organic compounds) or via the required derivatization reactions (such as metal ions after reaction with suitable hydrophobic ligands) in the starting solution. can be extracted and pre-processed from concentrated.

CPE is divided into three steps: The first three stages are the solubilization of the analyte into micellar aggregates, clouding, and phase separation for analysis. A surfactant solution swiftly separates into two different phases when heated to a critical temperature. While the other phase contains surfactant at a concentration below or equal to the critical micelle concentration, one phase is a surfactant-rich phase (Ojeda & Rojas, 2009). In the surfactant-rich phase, hydrophobic molecules that were previously bound to micelles in solution are released. This impact is noticeable, especially with respect to polyoxymethylene surfactants. The two ethylene oxide fragments in the micelle, which when hydrated repel one another at low temperatures and attract one another at higher degrees due to

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dehydration, are responsible for this phenomenon. The theory and related applications of this extraordinary separation technique have been discussed in various reviews and reports on its use in metal determination (McIntire, 1990; Hinze & Pramauro 2006; Sanz-Medel et al., 1999; Quina & Hinze, 1999; Stalikas, 2002; Rubio & Perez-Bendito, 2003; Sosa-Ferrera et al., 2004; Paleologos et al., 2005; Bezerra et al., 2005; Silva et al., 2006; Madej, 2009).

Recently, CPE was marketed as an attractive alternative to liquid-liquid extraction. This approach is based on the observation that most non-ionic surfactants form micelles in aqueous solution and become cloudy when heated to their cloud point temperature (Watanabe & Tanaka, 1978). To separate the two phases, the surfactant-rich phase and the aqueous supernatant phase, in CPE, the appropriate experimental conditions must be met, with the surfactant concentrated near to the critical micelle concentration (cmc). As a result, temperature change causes two-phase separation in zwitterionic and non-ionic surfactant solutions, whereas other factors cause two-phase separation in ionic surfactant solutions (such as pH, the addition of an ionic salt or an organic solvent). Parameters that produce phase separation can limit the types of compounds that can be extracted.

As a result, weakly basic compounds (like many drugs) which are ionized in low pH solutions, cannot be analysed using acidic solutions, while thermos labile chemicals (such as several vitamins) cannot be analysed using CPE at high temperatures. Figure 1 illustrates the actions needed in each of the five crucial CPE periods (Bişgin, 2018): (I) treating the sample with a surfactant, (II) keeping the temperature at a tolerable level for a while, (III) centrifuging, (IV) removing the supernatant and (V) correctly handling the surfactant-rich phase.

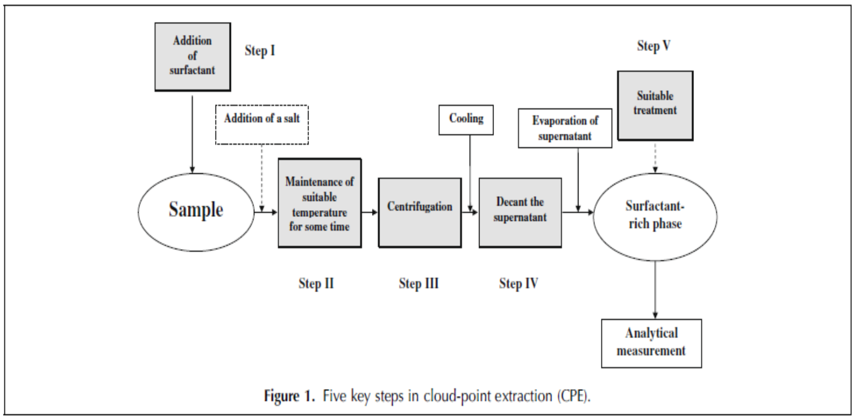

Figure 1. Five key steps in cloud-point extraction (CPE).

Fig. 1. Five key steps in cloud-point extraction (CPE)

If these techniques don't work, you'll need to perform additional cleanup (such solid-phase extraction (SPE) of the extract being studied) or switch to another detection method. Ionic strength, temperature, and the kind and concentration of the surfactant are the three most important key variables. When developing a CPE approach, these considerations must be considered. The acidity of the sample solution, like liquid-liquid extraction (LLE), is one of the most important factors controlling how well the analyte partitions in the concentrated surfactant-rich phase. The efficiency of maximum extraction of ionized species has been achieved at pH values where the uncharged form of the sample predominates. In micelle-mediated extraction, Various surfactants can be used. The three types of surfactants that are most frequently utilized are anionic, double-ionic, and non-ionic.

In general, the effectiveness of CPE increases with the introduction of more hydrophobic surfactants and hydrophobic analytes. The surfactant amount present in the sample solution is an important additional factor affecting the CPE effectiveness. of CPE. The recommended range for surfactant concentrations is a narrow band. As the final surfactant volume increases, it is seen that the analytical signal degrades over the optimal range. However, analytical accuracy and reproducibility deteriorate if surfactant concentration is dropped below the values that are recommended.

When the temperature increases, dehydration occurs and the volume of the surfactant-rich phase decreases. Studies have shown that two phases, a water micelle phase and a micelle-rich phase, must be maintained during CPE for a period and must be performed at temperatures above the cloud point temperature. Using ionic compounds creates a repulsive electrostatic effect. This can be explained by the fact that increasing ionic strength usually promotes phase separation and improves recovery. The effect of ionic strength on biological fluids is particularly significant (Bişgin, 2018).

Spectrophotometry is the most popular and enticing approach since it is simple and less expensive to use than other instrumental processes (Bişgin et al., 2015). The measurement of dyes using spectrophotometry can be difficult because of the low dye concentrations and matrix interference in real samples (Karatepe et al., 2017). As a result, many analytical methods such dispersive liquid-liquid micro extraction (DLLME), CPE, and others are combined with UV-Vis spectroscopy (Pourreza & Zareian, 2009; Bazregar et al., 2018; Yu & Fan, 2016).

Solid-phase extraction (SPE) has been developed to identify food dyes. For the identification and quantification of trace hazardous chemicals in diverse matrixes, such as dyes and heavy metal ions, CPE, a separation, purification, and enrichment approach, has been widely used (Li et al., 2017; Heidarizadi & Tabaraki, 2016; Nambiar et al., 2017; Shi et al., 2004). Phase separation makes it possible to observe two stages in CPE (Lemos et al., 2007). The crucial temperature over which the surfactant-containing solution turns turbid and divides into two phases is known as the cloud point temperature. First, the target analyte is present in very small quantities in the surfactant-rich phase. The diluted aqueous phase, which is at the second stage and has a large volume (Surme et al., 2007).

The spectrophotometric technique combined with CPE offers appealing qualities in regular examinations of metals and chemical compounds in various matrices as an alternative to using the pricy instruments mentioned above (Candir et al., 2008; Khammas et al., 2014; Khammas, Zet al., 2013; Pincemaille, et al., (2018). The unaided eye is the best tool for finding cloud patches. It is especially suitable when the appearance of the solution changes quickly and noticeably. The cloud point temperature is the point at which a solution begins to get clouded (cloud). However, since eye observation is subject to observer error, especially if cloudiness is increasing, its assessment is essentially arbitrary (Xiaoman et al., 2020).

An aqueous two-phase system "ATPS" is used according to standard CPE procedures. It was prepared by heating and centrifuging a PEG-based aqueous solution containing a surfactant or mixture of surfactants to produce a surfactant-rich phase containing the target analyte. In this study, temperature-induced cloud point extraction (TICPE) and a new methodology were used to extract, separate, or elevate flavonoids from Euonymus alatus (see Figure 1). To do this, flavonoids extracted from euonymus by ultrasonically-assisted extraction (UAE) using PEG-water as a homogeneous media system. (Magda et al., 2021).

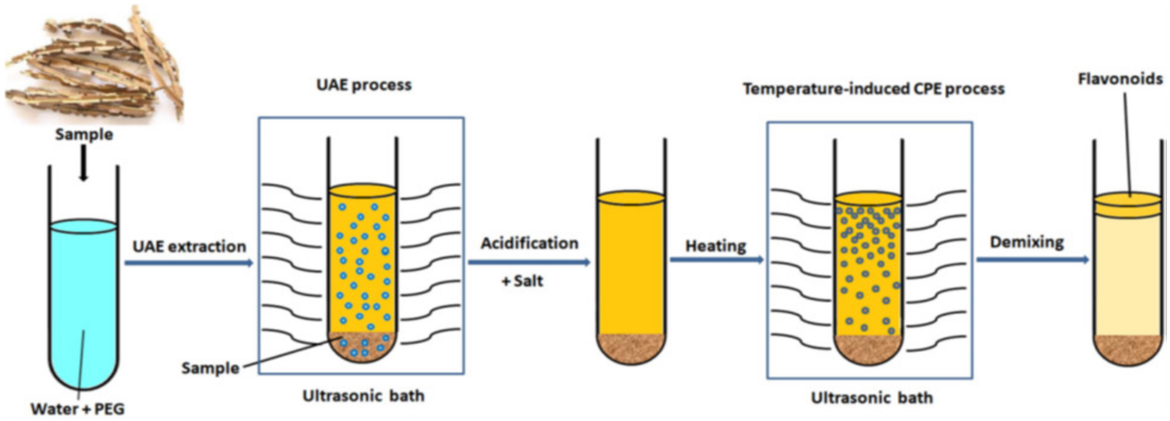

Fig. 1. A method for the extraction and separation of flavonoids from euonymus alatus by TICPE-tagged UAE

The CPE method has gradually replaced the conventional liquid-liquid extraction process as the standard. Since CPE uses very small volumes of non-hazardous surfactants rather than dangerous organic solvents, it is far healthier and more environmentally sustainable than other kinds of separation processes. CPE is an appealing method that lowers exposure and solvent consumption, which reduces disposal costs and the amount of time required for pre-concentration of metal ions following the production of sparsely water-soluble complexes (Ghasemi & Kaykhaii, 2015). To deal with these problems, the micro-cloud point extraction (MCPE) method was put out. The advantages of CPE are maintained, however MCPE completely does away with the water bath stage and increases the amount of dangerous chemical solvents utilized from a few mL to a few 10 L. They make MCPE an efficient, inexpensive, and ecologically friendly process (Ghasemi & Kaykhaii, 2016).

Advantage of CPE

CPE provides several advantages over traditional liquid-liquid extraction. Instead of using a lot of dangerous and flammable organic solvents like traditional solvent extraction does, CPE uses water. Surfactant's ability to lower analyte losses due to its adsorption onto the container can also increase CPE's recovery efficiency and pre-concentration factors. Metal ions have been separated and pre-concentrated in a variety of substances employing CPE prior to their detection by analytical techniques. The new online deployment of CPE into an FIA system has considerable benefits because it does away with all manual work (Ojeda & Rojas, 2009).

The CPE technique (green method) has become the best extraction method due to its advantages over other extraction techniques, including its simplicity, safety, affordability, good recovery, high enrichment factor, low consumption of organic solvents, ability to extract a variety of compounds and metals, as well as its ease of use and high efficiency (Katsoyannos, et al., 2012). Specifically in the extraction and pre-concentration processes, analytical procedures are increasingly using micelles and surfactants. CPE is an environmentally friendly extraction technique when compared to other liquid-liquid extraction techniques. The use of minute amounts of extracted solvents or surfactants in place of toxic organic solvents allowed for the development of CPE safer procedures and the measurement of a variety of analytes, including organic, inorganic, and nano-compounds (Shariati & Golshekan, 2014; Majedi et al., 2012; Abdullah, 2017). Additionally, less solvent is being consumed, which reduces extraction times and costs (Tabrizi, 2006). Medicinal substances have been extracted and identified using CPE from a range of matrixes.

Hayati et al., (2006) proposed employing cloud point extraction and spectrofluorimetry as a quick and efficient method to evaluate thiamine levels in human urine. By oxidizing thiamine with ferricyanide, thiochrome is produced, which is then extracted into Triton X-114 micelles for spectrofluorimetric measurement. To measure paracetamol using spectrophotometry, cloud point extraction (CPE) was used (PCT). The process is based on the acidic hydrolysis of PCT to PAP, which then combined with tetrahydroxy-calix[4]arene (CAL4) and an oxidizing agent (KIO_4) to produce a blue colored product at room temperature. Following formation, the PAP-CAL4 blue dye was captured in Triton X-114's surfactant micelles and detected at 590 nm. (Khammas, 2009: Mashhadizadeh & Jafari, 2010).

According to Zuhair (2015) the CPE methodology offers various advantages to those using extraction/preconcentration in analytical chemistry and research. These advantages include robustness, low cost, excellent extraction efficiency, and the ability to use a novel, exciting green chemistry process. Additionally, the ability to conduct online analysis using most instrumental approaches will create a desirable alternative in the field of automated separation techniques. Even though there have been numerous developments in linking CPE with instrumental approaches, there will be even more advancements in this field of study during the coming years. To fully comprehend the operation of the parameters governing the behaviour of micelle-mediated extraction, additional theoretical foundations pertaining to the process of separation and preconcentration are also required.

By creating a charge transfer-ion pair complex with bromothymol blue in an acetate buffer medium, codeine was identified. Triton X-114 was used to extract the compound, and an absorbance measurement at 430 nm was taken. (Zuhair, 2015). In an acidic solution, trifluoperazine hydrochloride (TFPH) formed a coloured complex with vanadium (V-TFPH), which was then extracted by the surfactant Triton X-114 and spectrophotometrically quantified at a wavelength of 476 nm. (Khammas & Mubdir, 2015). In dilute acidic medium, norfloxacin reacts with Fe(III) ions to form coloured hydrophobic (Fe(III)-NOR) complex. This complex is first extracted as a mediated extractant in micelles of Triton X-114. This is done after NOR and Fe(III) ions are separately measured spectrophotometry at 432 nm. (Khammas & Mubdir, 2015).

Aspirin was extracted and preconcentrated using Triton X-114, a non-ionic surfactant, in the presence of HNO3. At 305 nm, the absorbance of this mixture was measured in comparison to a blank. (Katsoyannos et al., 2012). Amoxicillin and Metoclopramide hydrochloride concentrations in medicines were determined using a method that was provided. The method involves the reaction of diazotized Metoclopramide and Amoxicillin in alkaline medium to produce an orange coloured and water-soluble product that is readily extractable from micelles in nonionic surfactant (Triton X-114). The two drugs are measured consecutively at 479 nm. (Sarsam & Theia'a, 2024).

Using spectrophotometry, the three different cephalosporin classes of Cefotaxime (CFX), Ceftriaxone (CFT) and Cefepime (CFM) were identified. The procedures involved creating an azo dye by reacting diazonium salts of CFT, CFX, and CFM with phenol, resorcinol, and α-naphthol in basic solution. The resulting compounds were then extracted using Triton X-114 and measured at 545, 500, and 515 nm, respectively (Dhahir & Mahdi, 2017). Following diazotization and coupling with o-aminobenzoic to produce azo dye, lamotrigine was identified. After that, Tritone X-114 was used to extract the substance, and its absorbance was measured at 424 nm (Hafez, 2017). In an acetate buffer media with a pH of 3.5, bromophenol blue and vardenafil HCl formed an ion pair combination. The complex was extracted using Triton X-114, and the surfactant-rich phase from that extraction was diluted with methanol before its absorbance at 420 nm was measured (Ulusoy & Akçay, M. (2018).

Using the cloud point extraction (CPE) methodology, a spectrophotometric approach for clonazepam (CLO) determination in pharmaceutical dosage forms was described. The procedure uses Triton X-114 as a surfactant to recover the product from the oxidative coupling between reduced CLO and phloroglucinol (PHG), which is then measured at 543 nm (Tabrizi, 2006). For the measurement of catecholamine medications, Hassan and Mizher (2018). established batch and cloud point spectrophotometric approaches. Cloud-point extraction technique to get the most analytical data and to obviate any potential measurement interference. The batch approach depends on diazotizing 2-aminothizole and combining it with Dopamine or Adrenaline. The violet-coloured dye with Adrenaline has λ_{max} at 565 nm, and faint violet coloured dye with Dopamine at 555nm. The UV Visible spectrophotometry measures the separation and preconcentration of violet dye in the cloud point extraction procedure. The batch method's analytical results include concentration ranges of 1.0 - 17.5 and 1.0 -12.5 µg/ml with molar absorptivity values of 1.7 x 10^4 and 5.51 ×10^5 l.mol^{-1}.cm^{-1}, Sandell's sensitivity values of 0.0175 and 0.061 µg/cm^2, limit of detection is 0.043 and 0.038 µg/ml and RSD is 0.65% and 0.91% for Adrenaline and Dopamine respectively.

The concentration range for the CPE method was 0.25 to 5.0 µg ml^{-1} with molar absorptivity values of 4.8×10^4 and 1.8×10^5 l.mol^{-1}.cm^{-1} for Adrenaline and Dopamine, respectively, Sandell's sensitivity values were 6.1 × 10^{-3} and 0.01 µg/cm^2, limit of detection was 0.019 and 0.025 µg/ml, RSD is 0.307 and 0.445, and recovery% were 100.03% and 99.93% respectively. The measurement enrichment factors for Dopamine and Adrenaline are 2.71, 2.46, as well as the preconcentration factor was 25. Adrenaline and Dopamine measurement in conventional medications and urine using the two approaches was successful. alongside catecholamine. Additionally, cloud-point extraction method was used to invest the coloured dye result from the reaction to collect the most analytical data and to obviate any potential measurement influence. These techniques have been used successfully to estimate catecholamine molecules in medicines and urine.

For the determination of vitamins B1 (thiamine) and B2 (riboflavin) utilizing cloud point extraction-HPLC technology, trace levels based on cloud point extraction were disclosed. The method is based on the complex formation between each vitamin and Ag (I) ions that were removed to non-ionic surfactant phase cetyl peridinum chloride (CPC) in the presence of KNO_3, Tergitol, and Genapol. (Dhahir & Mohammed, 2019). After being diazotized and combined with 4-tert-butylphenol and 2-NPT in basic medium, cefdinir was identified. Triton X-114 was used to remove the azo dyes, and the measurements for each reagent were made at 490 and 535 nm, respectively (Abood, et al., 2019). A cloud point extraction approach and a spectrophotometric method are both used to determine the presence of sulphadimidine sodium in Trtion X-114. The procedure involved diazotizing the medication Sulphdimidine Sodium, combining it with α-naphthol in the basic medium to produce an orange-coloured product, and then extracting it using a surfactant to measure it at 473 nm. (Dhahir & Mohammed, 2019).

Streptomycin Sulfate was measured using the cloud point extraction method with Trtion X-114. The procedure involved creating an Ag+1 chelating complex at pH 12 and measuring it at 404 nm. (Shihab & Al-Sabha, 2020). In a study by Wahdan et al., 2021), three spectrophotometric methods have been proposed for the determination of Methyldopa and Salbutamol. The first method involved an oxidation of Methyldopa by ferric nitrate and then coupling with thiosemicarbazone, yielding a dark green coloured product with an absorbance maximum at 460 nm. In the presence of potassium iodide and sodium hydroxide, salbutamol undergoes a coupling reaction with 4-nitrophenyl hydrazine to produce a violet-coloured substance with a maximum 530 nm absorbance. Beer's law is seen between 1 and 50 µg/ml with sandall's sensitivity of (0.039,0.073), and molar absorptivity of 0.536×10^4 and 0.327×10^4 l.mol^{-1}.cm^{-1} for Methyldopa and Salbutamol, respectively. The second technique is CPE for estimating the traces of above medicines that result from the same reaction. This technique allows for accurate drug assessment under the best experimental circumstances, with maximal absorption at 470 and 535 nm, respectively.

For Methyldopa and Salbutamol, the concentration range was between 0.25 and 6 µg/ml, the molar absorptivity values are 0.510×10^5 and 0.483×10^5 respectively, and the enrichment factor was between 9.51 and 14.72. The third method is based on using flow injection analysis (FIA) depending on measuring the absorption signal for products produced by the above reaction, to determine the phenolic drugs content. The stability of the product's colour was also studied. Active material for Salbutamol and Methyldopa was detected at maximum wavelengths of 460 and 530nm for each respectively. A total flow injection of 1.5 ml/min was pumped. The provided approaches were successfully used in this case to determine the presence of Salbutamol and Methyldopa in pharmaceutical formulation.

Three spectrophotometric methods for the determination of Cefixime drug, in its pure form and pharmaceutical formulations, have been described. In the method (I), NH_2 in Cefixime was diazotized and coupled with bisphenol A in basic medium, producing an orange-coloured product had a maximum absorbance at 490 nm. Beer's Law followed the concentration range of 1 to 50 µg/ ml with limit of detection of 0.157 µg/ml, and the molar absorptivity was 0.866×10^4 l.mol^{-1}.cm^{-1}. In method (II), CPE was used to pre-concentrate the Cefixime azo dye for trace amounts. The azo dye was measured at λ_{max} 500 nm. A calibration plot had a molar absorptivity of 0.961×10^5 l.mol^{-1}.cm^{-1}, and the concentration ranging from 0.25 to 6 µg/ml. Method (III) was based on the above reaction using FIA and the absorbance of the diazotized orange coloured product was measured at 490 nm.

Salbutamol and Methyldopa were determined in both pure and pharmaceutical formulations using a quick and efficient cloud point spectrophotometric method. The procedure was based on the formation of an ion-pair complexes with eosin Y dye. The extraction of complexes driven to Triton X-114 surfactant and measured at 558 and 564 nm for above drugs respectively. A rapid and precise approach for enriching and identifying Oxymetazoline (OMZ) in its pharmaceutical matrix was proposed by Wahdan, et al., (2021), using the CPE methodology. The method is based on the diazotization of OMZ and coupling with Metoclopramide in basic medium, The azo dye product was extracted by triton x-114 surfactant and measured at 510 nm.

However; applications of the CPE technique for determination of drugs is described in Table (1).

Table 1
Applications of CPE technique for determination of drugs

Analyte	λ max nm	Linear range	Surfactant	Molar absorptivity L.mol^{-1}.cm^{-1}	RSD %	Application
Thiamine		0.78 , 2.5-1000 ng mL^{-1},	Triton X-114		2.42	human urine
p-aminophenol (PAP)	590	1.5-12 mg.mL^{-1}	Triton X-114		2.15	Tablets and blood samples
	430	100-700 ng.mL^{-1}	Triton X-114		2.15	tablets and blood samples
Trifluoperazine hydrochloride (TFPH)	476	0.5-10 µg mL^{-1}	Triton X-114		0.48-3.40	pharmaceuticals and the spiked serum samples
Norfloxacin (NOR)	432	2.5-125 µgmL^{-1}	Triton X-114		0.04-0.66	biological and pharmaceutical samples
Aspirin	305	0.05–2 mg.L^{-1}	Triton X-114			pharmaceuticals
AMX and MCP	479	0.3-3.0 µgmL^{-1}	Triton X-114		2.35-10.8and 0.20-3.43	pharmaceuticals
ceftriaxone (CFT), cefotaxime (CFX), and cefepime (CFM)	545, 500, and 515	1 of 2.5 to 62.5 mg L	Triton X-114			Pure and pharmaceutical formulations
Lamotrigine drug	424	0.5–18 µg mL^{-1}	Triton X-114	2.88×10^3	1.62– 3.04	
vardenafil HCl (VARD)	420	0.1-2.0 µg mL^{-1}	Triton X-114		1.80	tablets
Clonazepam (CLO)	543	0.3-25 µg/mL	Triton X-114		2.5	tablets
catecholamine	565	1.0 – 17.5 µg/mL		4.8 x 104	0.307	Urine and drugs
vitamins B1 (thiamin) and B2 (riboflavin)		4 - 400 ng.mL^{-1} 1-500 ng.ml^{-1}			2.90 and 2.20	baby foods, milk, and cereals.
cefdinir (CFD), cephalosporin species	490535	3-100 µg/mL	Triton X-114	0.6129×10^{4,} 0.3361×10^4		commercial formulation
sulphadimidine sodium (SDMS)	473	1-12 µg·mL^{-1}	Triton X-114		1.3-5.28	pharmaceutical dose
Streptomycin Sulfate	404	2.5-30 µg·mL^{-1}	Triton X-114		good	some Pharmaceuticals
Methyldopa and Salbutamol	460,530	1-50) µg/ mL		0.536×10^4, 0.327×10^4		pharmaceutical preparation.
cefixime	490	1-50 µg mL^{-1}		0.866×10^4		bulk drug and pharmaceutical formulations
Salbutamol Sulphate and Methyldopa	558, 564	0.1-20, 0.3-10 µg mL^{-1}	Triton X-114	4.0×10^4 5. 7×10^4	<1.6 >0.3	pharmaceutical preparations.
Oxymetazoline	510	0.05-17.0 µg mL^{-1}	Triton X-114	2. 7×10^4	4.48	commercial nasals

CONCLUSION

This review has shown that it is possible to determine of the drugs that involved in the chemical reactions by using a combined CPE-spectrophotometry with high sensitivity, good recovery and good extraction efficiency. The CPE method is considered as green chemistry because the surfactants are not toxic, not flammable and not volatile. CPE is depended on liquid-liquid extraction technique using non-ionic surfactant such as Triton x-114 for preconcentration of the complexes or products because of chemical reactions.

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Competing Interest

The authors had no competing interests.

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